J. Clin. Chem. Clin. Biochem.
Vol. 20, 1982, pp. 61–63

A Case of Rare Heterozygous α_1-Antitrypsin Phenotype: IS

By *K. Bencze* and *X. Baur*

Institut und Poliklinik für Arbeitsmedizin (Director: Prof. Dr. G. Fruhmann), Pulmonary Section (Head: Prof. Dr. G. Fruhmann), Medical Clinic I (Director: Prof. Dr. G. Riecker), Klinikum Großhadern, University of Munich

(Received July 14/October 16, 1981)

Summary: We describe a case of the heterozygous antitrypsin phenotype IS. The 20 years old female employee had been recurrently suffering from cutaneous vasculitis over a period of three years. Serum trypsin inhibitor was found to be high (1394 ImU) during the acute stage of the disease. No other serological or physical abnormalities were detected. A relationship between certain Pi (the term Pi indicating protease inhibitor = α_1-antitrypsin) alleles and vasculitis is possible but not certain, as three out of four other patients with this disease were MM phenotypes, the other one being MS type.

Fall eines seltenen heterozygoten α_1-Antitrypsin-Phänotyps: IS

Zusammenfassung: Wir beschreiben einen Fall des heterozygoten Phänotyps des α_1-Antitrypsins IS. Die 20 Jahre alte Patientin erkrankte in den letzten drei Jahren in unregelmäßigen Abständen an einer cutanen Vasculitis. Die Serumhemmkapazität gegen Trypsin war während der akuten Phase der Krankheit hoch (1394 ImU). Es wurden keine anderen serologischen oder physischen Abnormitäten beobachtet. Eine Beziehung zwischen besonderen Pi(= Proteaseinhibitor = α_1-Antitrypsin)-Allelen und Vasculitis ist möglich, aber nicht sicher, da von vier anderen Patienten mit dieser Krankheit drei zu den MM-Phänotypen und einer zu den MS-Typen gehörten.

Introduction

It is well known that individuals homozygous with respect to the α_1-antitrypsin allele Z have an increased risk of panacinar emphysema, chronic obstructive pulmonary disease, liver cirrhosis and malignant hepatoma (1–5).

Reports concerning the pathological role of the heterozygous α_1-antitrypsin MZ and MS variants are controversial; some studies suggest a relationship to pulmonary diseases, others do not (6–11). There is evidence that heterozygosity may become a deciding pathological factor in the presence of additional pulmonary risks like smoking, exposure to industrial dusts, fumes and/or individual predisposition, e.g. abnormal enzyme patterns (10, 11).

From the genetic point of view it is possible for 25% of children from heterozygous parents to have the homozygous SS or ZZ type, whereas the other 50% will be in danger having heterozygous variants (9).

As opposed to the MZ and MS types, which have been the subject of several studies, certain rare variants can be described only in the form of case reports.

Case History and Results of Laboratory Investigations

For the last three years the 20 year old female employee has suffered recurrently from raised, erythematous cutaneous lesions, which developed simultaneously on average once to four times every 6 weeks. These eruptions were predominantly localized symmetrically on the lower extremities and were accompanied by marked swelling of feet, malleolar regions and lower legs. They also disappeared spontaneously within a week without treatment, or even within few days if corticosteroids were administered. Skin biopsy specimens showed mixed cellular infiltrate of blood vessels (neutrophils, lymphocytes and monocytes), nuclear debris and erythrocyte extravasation. In direct immunoflourescence studies there was strong evidence for deposition of immune complexes in vascular lesions (deposition of C 3, IgM and fibrin in the walls of superficial dermal venules).

Immune complexes, antinuclear antibodies, or rheumatoid factors were not detectable in the serum; C 3, C 4, C 5, C 1 inhibitor, bilirubin, prothrombin, aspartate aminotransferase, alanine

0340-076X/82/0020-0061$02.00

aminotransferase, γ-glutamyltransferase, alkaline phosphatase and cholinesterase, and erythrocyte sedimentation rate were within normal ranges.

Extensive clinical and laboratory investigations including pulmonary function tests suggested no further organic abnormalities, except chronic tonsillitis. The etiology of the disease remained unknown; intracutaneous and epidermal skin tests with a battery of allergens were negative. Interruption of oral contraceptives, the only drugs the patient took, led to no changes in the symptoms described.

Methods

The α_1-antitrypsin phenotypes were determined by isoelectric focusing on polyacrylamide gel slabs. As previously described (12), 20 µl of each serum was added to specially prepared gel slabs of 1 mm thickness.

Serum trypsin inhibitory capacity

Quantitative functional analyses of serum α_1-antitrypsin were performed according to the method of *Fritz* et al. (13). One inhibitor milliunit (ImU) is defined as reduction of the trypsin-catalyzed hydrolysis of the substrate N-α-benzyl-*L*-arginine-*p*-nitroanilide by 1 µmol per min ($\Delta A = 0.00332$ in 3 ml solvent).

Results

Using M_1M_2 standardized sera and reference sera of subjects with M_1S and M_1I phenotypes the rare phenotype IS was identified (fig. 1). Additional support for these results was obtained by a family study, which showed that the parents of the patient have M_1I and M_1S phenotypes (fig. 2). Serum trypsin inhibitory capacity was normal in the mother's serum (1145 ImU; normal range for IM-types 1150 ± 180 ImU), decreased in the father's serum (783 ImU; normal ranges for MS-types: 1060 ± 180 ImU), but rather high in the serum of the above mentioned patient (1394 ImU).

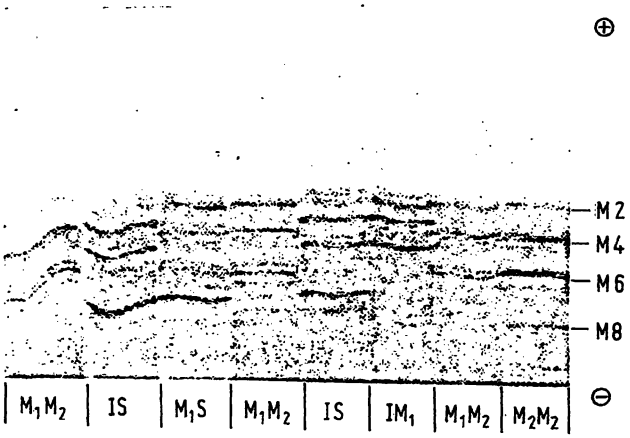

Fig. 1. Phenotyping of α_1-antitrypsin variants IS, MS and MI on polyacrylamide gel.

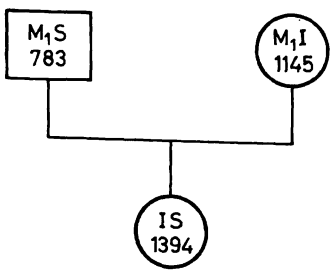

Fig. 2. Family pedigree of propositus protein inhibitor IS: phenotypes and serum trypsin inhibitory capacities.

Discussion

Many studies on the distribution of α_1-antitrypsin phenotypes in different populations have been published. Based upon the mean gene frequency from the studies mentioned in table 1 we calculated a frequency of the heterozygous variant IS of 8.6 in 100 000.

Tab. 1. Pi allele frequencies in some European and non European populations.

Ref. No.	Year	Country	Observed	Gene frequencies S	I	Z
8	1975	England	4042	0.0500	0.0000	0.0141
15	1972	France	1520	0.0679	0.0006	0.0590
16	1975	France	394	0.0660	0.0013	0.0216
17	1977	France	1653	0.0713	0.0036	0.0142
18	1978	France	560	0.0750	0.0018	0.0232
19	1970	Germany	516	0.0213	0.0010	0.0087
20	1977	Germany	538	0.0370	0.0060	0.0240
11	1980	Germany	280	0.0300	0.0018	0.0140
21	1975	Netherlands	1474	0.0160	0.0014	0.0123
22	1977	Netherlands	708	0.0297	0.0035	0.0049
19	1970	Hungary	172	0.0174	0.0029	0.0145
23	1975	Ireland	1000	0.0385	0.0030	0.0200
24	1978	Italy	202	0.0297	0.0025	0.0099
25	1978	Italy	500	0.0670	0.0020	0.0150
26	1967	Norway	1967	0.0230	0.0012	0.0157
27	1979	Norway	1268	0.0270	0.0008	0.0246
28	1976	Portugal	330	0.1152	0.0015	0.0182
29	1977	Somalia	347	0.0144	0.0014	0.0072
30	1968	Spain	378	0.1124	0.0013	0.0119

It remains to be proven if there is a relationship between IS type and other Pi types on the one hand and the occurrence of immune complex vaculitis on the other hand. In order to clarify this question, screening studies of patients with this disease seem to be necessary. Our preliminary studies showed that three out of four patients with strong evidence for immune complex vasculitis (identification of deposits of immunoglobulins and C 3 within and around venules of dermal lesions) were of the MM type and the fourth one of this group belonged to the M_1S type. Furthermore, three out of the latter group also had significantly elevated serum trypsin inhibitory capacities (> 1499 ImU).

It is well known that α_1-antitrypsin behaves as an acute phase reactant and that it's serum concentration increases during inflammation. This may be the cause of the elevated serum trypsin inhibitory capacity values in patients with acute stages of vasculitis.

References

1. Sharp, H. L., Bridges, R. A., Krivit, W. & Freier, E. F. (1969) J. Lab. Clin. Med. *73*, 934–939.
2. Mittman, C., Liebermann, J., Marasso, F. & Miranda, A. (1971) Chest *60*, 214–221.
3. Berg, N. O. & Erikson, S. (1972) New Engl. J. Med. *287*, 1264–1267.
4. Kueppers, F. & Black, L. F. (1974) Am. Rev. Resp. Dis. *110*, 176–194.
5. Liebermann, J. (1976) N.Y. State Med. *76*, 181–186.
6. Buist, A. S., Sexton, G. J., Abdul-Mordy, H. Azzam & Adams, B. E. (1979) Am. Rev. Respir. Dis. *120*, 759–766.
7. McDonough, D. J., Nathan, S. P., Knudson, R. J. & Lebowitz, M. D. (1979) J. Clin. Invest. *63*, 299–309.
8. Cox, D. W., Hoeppner, V. H. & Levison, H. (1976) Am. Rev. Respir. Dis. *113*, 601–606.
9. Osswald, P. & Gathmann, H. (1979) Leber, Magen, Darm *9*, 241–246.
10. Evans, H. E. & Bognacki, N. (1979) Envir. Health Persp. *29*, 57–61.
11. Bencze, K., Sabatke, L. & Fruhmann, G. (1980) Chest *77*, 761–763.
12. Bencze, K. & Sabatke, L. (1980) J. Clin. Chem. Clin. Biochem. *18*, 13–16.
13. Fritz, H., Trautschold, I. & Werle, E. (1970) in Methoden der enzymatischen Analyse, pp. 1023–1029 (Bergmeyer H. U., ed.) Verlag Chemie; Weinheim.
14. Cook, P. J. L. (1975) Ann. Hum. Genet. *38*, 275–289.
15. Robinet-Levy, M. & Rieunier, M. (1972) Revue Fr. Trasfus. *15*, 61–72.
16. Morin, T., Martin, J. P., Fedelman, G., Rueff, B., Benhamer, J. P. & Ropatz, C. (1975) Lancet *II*, 250–251.
17. Arnaud, P., Chapius-Cellier, C., Vittoz, P. & Creyssel, R. (1977) Hum. Genet. *39*, 63–68.
18. Sesboüe, R., Charlionet, R., Vercaigne, D., Giumbretiere, J. & Martin, J. P. (1978) Hum. Hered. *28*, 280–284.
19. Kellerman, H. & Walter, H. (1970) Hum. Genet. *10*, 145–150.
20. Genz, T., Martin, J. P. & Cleve, H. (1977) Hum. Genet. *38*, 325–332.
21. Hoffmann, J. J. M. L. & van den Broek, W. G. M. (1975) Hum. Genet. *32*, 43–48.
22. Klasen, E. C., Franken, C., Volkers, V. S. & Bernini, L. F. (1977) Hum. Genet. *37*, 303–313.
23. Blundell, G., Frazer, A., Cole, R. B. & Nevin, N. C. (1975) Ann. Hum. Genet. *38*, 289–344.
24. Klasen, E. C., D'Andrea, F. & Bernini, L. F. (1978) Hum. Hered. *28*, 474–478.
25. Piantelli, M., Auconi, P. & Musiani, P. (1978) Hum. Hered. *28*, 468–473.
26. Fagerhol, M. K. (1967) Acta Pathol. Microbiol. Scand. *70*, 421–428.
27. Gulsiuk, A. & Fagerhol, M. K. (1979) Scand. J. Respir. Dis. *60*, 267–274.
28. Martin, J. P., Sespone, R., Charlionet, R., Ropartz, C. & Pereira, M. T. (1976) Hum. Hered. *26*, 310–314.
29. Massi, G. & Vecchio, M. F. (1977) Hum. Genet. *38*, 265–269.
30. Fagerhol, M. K. & Tenfjord, O.W. (1968) Acta Pathol. Microbiol. Scand. *72*, 601–608.

Dr. K. Bencze
Institut und Poliklinik für Arbeitsmedizin
der Universität
Ziemssenstr. 1
D-8000 München 2